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TRANSACTIONS

Studies on Vitamin B₂ Complex.

IV—Effect of Carbohydrate on Vitamin B₂ Deficiencies.

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During the experiments on vitamin B₂ deficiencies, it was found that there was a marked difference in the nutrition of young rats receiving vitamin B₂ complex deficient diets containing corn-starch, as compared to other rats receiving comparable diets containing sucrose as the source of carbohydrate, which was probably due to the minute quantity of some factor (or factors) of vitamin B₂ held by the starch particles.

Morgan, Cook and Davison,⁽¹⁾ Mitchell and Dodge⁽²⁾ reported that high lactose ration had cataractogenic effect on rats, whereas Day, Langston and O'Brien⁽³⁾ who used the more usually employed corn-starch as the basal carbohydrate, showed that flavin deficiency in the ration produced cataract in rats. There are several reports that while cataracts were more or less regularly produced on lactose-containing diet, cataracts resulting from vitamin G deficiency were not observed. This led to the use of sucrose as well as of lactose and corn-starch with varying single and multiple B₂ deficiencies.

This paper reports the effects of the three basal rations with sucrose, corn-starch or lactose as carbohydrate constituent on the vitamin B₂ deficiency disease occurring in the rat.

EXPERIMENTAL.

The basal rations had the following composition: Purified fish protein 18 parts; carbohydrate (sucrose, corn-starch or lactose) 68 parts; crisco 9 parts; McCollum's salt mixture 4 parts. In some of the lactose diets a lower proportion of fish protein was used, 10 parts, but in total amount 20 parts by adding 10 parts of dried egg-white powder. The crisco content in the sucrose diets was reduced from 9 to 3 parts in the later experiments and 2 parts of powdered agar-agar were added. The lactose was purified by repeating extraction with hot 94 per cent alcohol. The corn-starch was exposed to strong day-light in thin layer

for 10 days before being incorporated in the diet.

Rats weighing 55 to 65 g were placed on the basal diets, provided daily with one drop of cod liver oil and 10~20 γ of vitamin B₁ according to body weight, care being taken in the group distribution as described in the preceding paper.⁽⁴⁾

Flavin, vitamin B₆, and filtrate factor, singly or in combination of the two or three, were used as the vitamin B₂ factors.

Flavin was given as egg-white powder at the level of 6 per cent, except 10 per cent in the lactose ration.

Vitamin B₆ and filtrate factor concentrates were fed in daily amounts per rat equivalent to 1 g dried yeast.

Preparation of Supplementary Materials.

Vitamin B₆ concentrate was prepared as follows: 500 g of dried brewer's yeast were extracted twice with 1600 cc of cold 70 per cent alcohol and three times with 1500 cc of boiling 80~85 per cent alcohol. The combined extracts were concentrated in vacuo to 500 cc, and the solid and the fats removed by filtering and ether extraction. The solution was then adjusted to pH 2.0 with H₂SO₄ and treated with 80 g of acid clay to remove vitamin B₁ and flavin. The first adsorbate was rejected, and the filtrate was treated twice with 85 g portions of acid clay. The second and third adsorbates, after being washed with water and alcohol, were eluted with 0.2 N Ba(OH)₂. The resultant solution was freed from barium by addition of H₂SO₄ and concentrated under reduced pressure (1 cc = 2 g yeast, dry wt.). The eluate was used as vitamin B₆.

Filtrate factor: The final filtrate, from the acid earth adsorbate of yeast extract mentioned above, was treated with Ba(OH)₂ to remove H₂SO₄ and concentrated in vacuo to 250 cc (1 cc = 2 g yeast, dry wt.).

The Effect of Lactose.

The outstanding feature in the groups on the lactose diet was the complete absence of dermatitis, frequent appearance of cataract, and slightly subnormal growth, but good nutritional condition as the control rats, even with the entire B₂ deficient diet. Young rats when first put on this ration often had a diarrhea which subsided later, although the stool frequently continued to be softer than that of rats on other carbohydrate diet.

By giving vitamin B₆ (it being understood that B₁ is present in all cases) no growth improvement was brought out. The addition of dried egg-white powder as flavin at the level of 10% to the low protein ration composed of 10 parts of fish protein, resulted in some increase of growth rate. When filtrate factor with the other two factors was given, the growth was, however, definitely stimulated and the appearance of cataract was somewhat delayed. Even with the administration of 4% dried brewer's yeast, at the beginning of the experiment, the development of cataract could not be prevented.

It is of interest that vitamin B₆ and flavin along with the 68% lactose diet

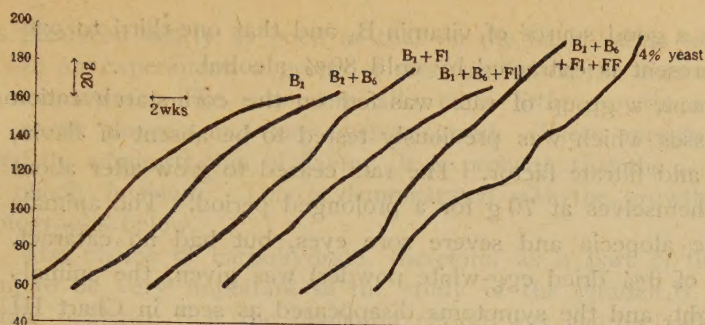


Chart 1. Average growth curves of rats on lactose diet with one, two, three or four of the vitamins: B₁, flavin (Fl), filtrate factor (F), vitamin B₆ (B₆).

the alcohol extract of ceca content of the lactose-fed rats on the rats depleted on the corn-starch diet.

The Effect of Corn-starch.

The growth of the rats on corn-starch diet without supplements was very slow, sometimes dermatitis developed but none of the animals has shown any cataract. By the addition of B₆ or flavin singly or in combination, the growth was not greatly stimulated, in spite of the fact that administration of flavin prevented alopecia, a denuded condition, called a "skin affection" by Chick, Copping, and Edgar.⁽⁶⁾ However, the addition of filtrate factor after growth had ceased on flavin gave a marked growth response and improvement of the symptoms; filtrate factor alone produced little improvement. This result confirmed also the previous finding.⁽¹³⁾ The phenomenal growth response resulting from the addition of flavin and filtrate factor to the corn-starch diet and the relative ineffectiveness of the addition of vitamin B₆ to flavin gave us the supposition that corn-starch carried appreciable amounts of vitamin B₆. Indeed recent reports of Dann⁽⁷⁾ and Copping⁽⁸⁾

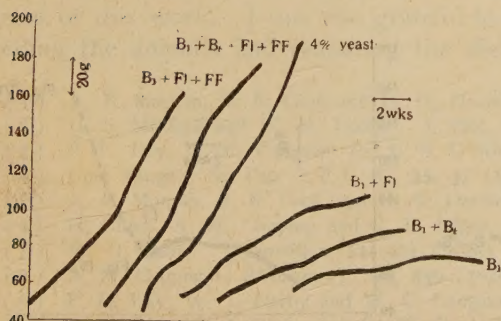


Chart II. Average growth curves of rats on cornstarch diet with one, two, three, or four of the vitamins: B₁, flavin (Fl), filtrate factor (FF), vitamin B₆ (B₆).

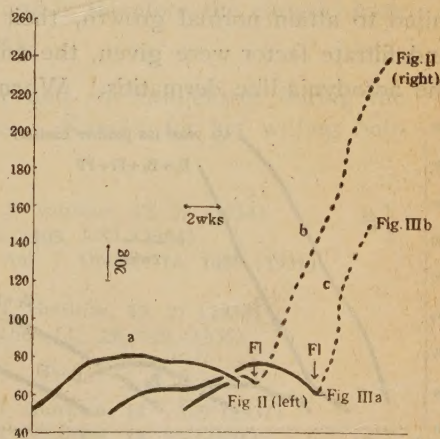


Chart III. Growth curves of rats on cornstarch diet containing 5% cane molasses, as source of filtrate factor and B₆. Fl (flavin).

brought about little improvement on the growth. This phenomenon may perhaps be attributed to the fact that lactose favours the production of both flavin and vitamin B₆ in the intestine probably by microorganisms as indicated in the experiment of Morgan and his associates⁽⁵⁾ with

showed that corn-starch is a good source of vitamin B_6 and that one-third to one-quarter of the vitamin B_6 present is extracted by cold 80% alcohol.

In an earlier experiment, a group of rats was fed on the corn-starch ration containing 5% cane molasses which was previously tested to be absent of flavin, but to contain vitamin B_6 and filtrate factor. The rats ceased to grow after about 4 weeks and maintained themselves at 70 g for a prolonged period. The animals showed very characteristic alopecia and severe sore eyes, but had no cataract. When flavin (at the level of 6% dried egg-white powder) was given, the animals immediately resumed weight, and the symptoms disappeared as seen in Chart III and the photographs (Figs. 2 and 3). The rat receiving flavin from the earlier stage of the experiment was also included for comparison. This was entirely different from the results of Day, Darby, and Langston⁽⁹⁾ who have consistently produced cataract on flavin deficient diet. We must realize, therefore, that cataract in rats resulting from flavin deficiency is apparently different in etiology from the cataract which results from lactose feeding, as pointed out by Day, Darby, and Cosgrove.⁽¹⁰⁾

The Effect of Sucrose.

The rats fed on the sucrose ration without supplement (it being understood that vitamin B_1 is present in all cases) showed little or no gain, and survived for only several weeks. The addition of flavin produced growth stimulation for some interval, but developed the acrodynia-like dermatitis due to the deficiency of vitamin B_6 . If this condition continued the rats would lose their weight and die. The survival period of the animals, however, was greatly influenced by the amounts of the fat contained in the basal ration, in other words, the fat exerts a remarkable sparing action on vitamin B_6 (the relationship between vitamin B_6 and fats will soon be reported).

Obviously the ration must be supplemented with one or more of the factors of vitamin B_2 complex. When vitamin B_1 and flavin were given together, the rats failed to attain normal growth, their weight being subnormal. When both flavin and filtrate factor were given, the animals continued to grow, but often developed the acrodynia-like dermatitis. When all three factors were given, normal growth

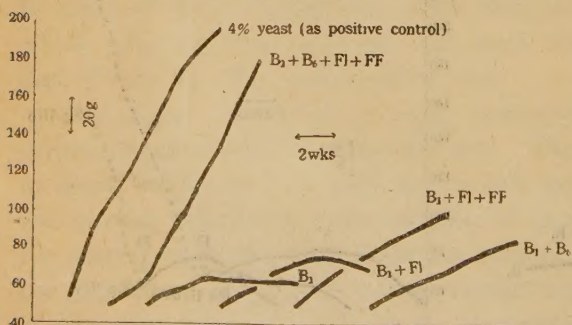


Chart IV. Average growth curves of rats on sucrose diet with one, two, three, or four of the vitamins: B_1 , flavin (Fl), filtrate factor (Fl), vitamin B_6 (B_6).

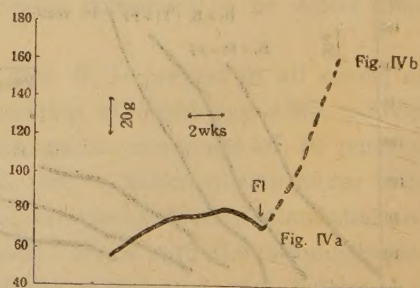


Chart V. Growth curves of rats on sucrose diet containing 5% cane molasses, as source of filtrate factor and B_6 . Fl (flavin).

was produced nearly as good as that on the basal ration with 4% yeast.

In an experiment, a group of rats was fed on the sucrose diet containing 5% cane molasses. The rats were allowed a little growth, no cataract developed, but severe alopecia and sore eyes occurred. These symptoms were immediately cured by administration of flavin. It is probable that the cane molasses was lacking chiefly in flavin. This is demonstrated with the growth curve as well as the photographs below.

The choice of carbohydrate, therefore, as a part of the basal ration would seem to be very important in the study of the vitamin B₂ deficiency. There is certain evidence that the depletion period was shorter and deficiency symptoms more severe with sucrose instead of corn-starch: moreover it was shown that the different influences of sucrose and dextrine on growth and dermatitis appeared in the rat.⁽¹¹⁾⁽¹²⁾

SUMMARY AND CONCLUSIONS.

1. Lactose had an unique effect on the nutrition of rats. None of the lactose-fed rats developed dermatitis, but cataract. The animals continued to grow even on the entire B₂ deficiency. Vitamin B₆, flavin or filtrate factor separately administered did not give any appreciable improvement on either growth or cataract. When all three factors, B₆, flavin, and filtrate factor, were supplied, a definite effect on growth was produced and slower development of cataract was brought about.

2. The rats fed on the corn-starch diet failed to grow and developed alopecia unless flavin and filtrate factor were supplied. Vitamin B₆ had little effect on improvement of the growth rate. This factor appeared to be present in the starch. When fed on the corn-starch diet containing 5% cane molasses, the rats maintained themselves at 70 g for a prolonged period and developed severe alopecia and sore eyes, but no cataract.

3. When sucrose was used as the basal carbohydrate, earlier and severer development of dermatitis and growth failure occurred unless vitamin B₆, flavin and filtrate factor were supplied. No cataract appeared on this diet. Apparently sucrose is completely free from all three factors and is therefore the carbohydrate of choice for the study of the vitamin B₂ deficiency.

I wish to thank Dr. U. Suzuki for his advice and encouragement during the progress of this work. I am also grateful to Miss T. Kaneko for her willing help in feeding the animals and preparing the diets.

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- (3) P. L. Day, W. C. Langston and C. S. O'Brien: *Am. J. Ophth.*, **14**, 1005 (1931)
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A. Normal



B. Rat with cataract resulting from the 68% lactose.

Fig. 1.



Fig. 2. Left—Rat fed on the corn-starch diet in flavin deficiency: alopecia and sore eyes, but no cataract.

Right—Rat fed on the corn-starch diet with flavin: normal growth. These rats were photographed on the seventy-seventh day of experiment, at which time they weighed 68 g and 250 g, respectively.



Rat—a, Showing alopecia and sore eyes resulting from flavin deficiency, but no cataract (60 g).



Rat—b, Showing recovery of the rat (a) by administration of flavin (151 g).

Fig. 3.



Rat—a, Showing alopecia and sore eyes resulting from flavin deficiency (70 g).



Rat—b, Showing recovery of the rat (a) by administration of flavin (162 g).

Fig. 4

ABSTRACTS**from****TRANSACTIONS published in JAPANESE**

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

**On the Stimulant for Cane Sugar Formation
in Plants. (II)**

(pp. 419~421)

By Tetutaro TADOKORO and Naomoto TAKASUGI.

(Biochem. Lab., Faculty of Sci., Hokkaido. Imp. Univ.; Received Apr. 7, 1939.)

**Über die Verschiedene Eigenschaften von
Aspergillusarten. (VI)**

(ss. 422~428)

Von T. TAKEDA und O. TAKEUTI.

(The Department of Industry, Government Research Institute, Taiwan, Nippon;
Received Apr. 19, 1939.)

**Researches on Bamboos as a Raw Material for
Pulp in Taiwan.**

(pp. 429~434)

By Minoru TUTIVA and Setuo HUKUHARA.

(Industrial Research Institute of Taityu; Received Apr. 9, 1939.)

**The Influence of Monochromatic Lights on the
Action of Enzymes.**

(pp. 435~444)

By Reitaro MURAKAMI.

(Agricultural College, Utunomiya; Received Jan. 31, 1939.)

**[Report XVII.] Especially on the Influence of Infra-red
Rays on the Action of Enzymes.**

(Part 1.) Yeast Saccharase.

To investigate the influence of infra-red rays on the action of the saccharase in the yeast, the author has employed "Vim Ray" red lamp. The preparation of the enzyme, the addition of the enzyme solution into the substrate, the determination of the end product by the action of the enzyme and other treatments were

carried out as in the author's previous papers [Report XII], but the filters at the front of the boxes containing test tubes lighted by "Vim Ray" red lamp were different.

The transmission wave lengths of white, infra-red pass and black screens used as the light filter were $> 5700 \text{ \AA.}$, $> 8000 \text{ \AA.}$ and none, respectively.

In this experiment, the action of the yeast saccharase was found to be promoted by infra-red rays. The enzyme under the rays containing both visible and infra-red was more active.

**[Report XVIII.] Especially on the Influence of Infra-red
Rays on the Action of Enzymes.**

(Part 2.) Yeast Amylase.

As a light source "Vim Ray" red lamp was employed. The light filters were the same as in the author's previous paper [Report XVII]. The preparation of the enzyme, the addition of the enzyme solution into the substrate, the determination of the end product by the action of the enzyme and other treatments were the same as in the author's previous paper [Report XIII].

In this experiment, the action of the yeast amylase was found to be promoted by infra-red rays, but the enzyme was more active under the rays containing both visible and infra-red.

**[Report XIX.] Especially on the Influence of Infra-red
Rays on the Action of Enzymes.**

(Part 3.) Yeast Proteinase.

As a light source "Vim Ray" red lamp was employed. The light filters were the same as in the author's previous paper [Report XVII]. The preparation of the enzyme, the addition of the enzyme solution into the substrate, the determination of the end product by the action of the enzyme and other treatments were carried out as in the author's previous paper [Report XIV].

In this experiment, the action of the yeast proteinase was found to be promoted by infra-red rays, but the enzyme under the rays containing both visible and infra-red was more active.

**[Report XX.] Especially on the Influence of Infra-red
Rays on the Action of Enzymes.**

(Part 4.) Yeast Lipase.

"Vim Ray" red lamp was employed as a light source. The light filters were the same as in the author's previous paper [Report XVII]. The preparation of the enzyme, the addition of the enzyme solution into the substrate, the determination of the end product by the action of the enzyme and other treatments were the same as in the author's previous paper [Report XV].

In this experiment, the action of the yeast lipase was found to be promoted by infra-red rays, but not so much as by the rays containing both visible and infra-red.

**[Report XXI.] Especially on the Influence of Infra-red
Rays on the Action of Enzymes.**

(Part 5.) Yeast Catalase.

As a light source "Vim Ray" red lamp was employed. The light filters were the same as in the author's previous paper [Report XVII], and the preparation of the enzyme, the addition of the enzyme solution into the substrate, the determination of the end product by the action of the enzyme and other treatments were carried out as in another previous paper. [Report, XVI].

In this experiment, the action of the yeast catalase was found to be promoted by infra-red rays, though not so much by the rays containing both visible and infra-red.

**Studies on the Utilization and Digestion by Silkworms
of the Carbohydrates Contained in
Mulberry Leaves.**

(pp. 445~458)

By K. KATO.

(The Sericultural Experiment Institute of Gifu Prefecture; Received Mar. 8, 1939.)

**Part X. Research on the content of carbohydrates
in mulberry leaves.**

The content of carbohydrates in mulberry leaves varied with kinds of mulberry trees, degree of growth and quantity of manure, etc. In this research, the largest value of total carbohydrate content is 10.60%, smallest is 1.62% and the arithmetical mean value is 4.72%.

**Part XI. The Relation of the sericultural harvest and
the carbohydrate content of mulberry leaves.**

The sericultural harvest was best when bred on mulberry leaves which have 3~4% sugar content at first instar, and 4~5% sugar content at second instar.

**Part XII. Devise of the apparatus for determination
of carbohydrate content in leaves.**

As the results of various experiments, I devised an apparatus for determination of carbohydrate content in leaves. This apparatus is accurate and convenient for use at any place not equipped as a chemical laboratory.

Studies on Alkaline Earth. I.

(pp. 459~472)

By Kinjiro KAWASE, Hogai KA and KOZO KAWAKAMI.

(The Institute of Scientific Research, Manchoukuo; Received Apr. 10, 1939.)

In the alkaline district of North Manchuria, a kind of edible salt, "earth salt" or "tou-en" is manufactured from earth.

The earth, raw material of the salt, is extracted with water, filtered and evaporated, and by these primitive processes the earth salt is manufactured.

After a study of the physical and chemical properties and field observations on various earth salts, the following conclusions were obtained.

(1) The original earth which is used by the natives for the preparation of the earth salt contains about 24.895% of water soluble matters.

(2) The cation of the chemical constituents of the water soluble matters contains mainly Na^+ (99%~62%), while the hygroscopic alkali spot contains chiefly Ca^{++} , Mg^{++} (35%).

The amount of anions (Cl' , SO_4'' , CO_3'' , HCO_3') varies in accordance with the outside conditions.

The surface deposits visible to the naked eye contain more SO_4'' than Cl' , while the soil 1 cm below contains more Cl' than SO_4'' .

The deposit near the lake and the reddish-brown deposit which is rich in CO_3'' , HCO_3' , on the contrary, contain more Cl' than SO_4'' . The earth collected by natives for the production of earth salt contains extraordinarily more Cl' than SO_4'' . With CO_3'' , HCO_3' no such particular properties can be seen as in the case of Cl' and SO_4'' . Moreover they can be distinguished from the sea-salt by the fact that they all contain considerable quantities of NO_3' , NO_2' .

The Utilization of the By-products of Soy-beans. (Part V)

(pp. 473~477)

By Yosaburo IWASA.

(Dept. of Food Chemistry, Osaka Municipal Hyg. Lab.; Received Apr. 15, 1939.)

Studies on Fumigants. Part I. The Reaction Products of Trichlorethylene and Nitric Acid. No. 1.

(pp. 478~482)

By M. KOHNO and T. SAKAI.

(Sankyo Chemical Laboratory, Osaka; Received Apr. 28, 1939.)

Studies on the Etherification of Ethyl Alcohol.

Part I. Preliminary Experiments.

Part II. Determination of Ether by Distillation

Curve.

Part III. Experiments using such Catalysts as
Activated Acid Clay and Others.

(pp. 483~495)

By Masawo HORI, Kunitaka OH-IKE, and Yosiro HUKUSIMA.

(Central Research Institute, Japanese Government Monopoly Bureau;

Received Apr. 27, 1939.)

Saponin in the Bark of *Schima liukiuensis*, Nakai.

(pp. 496~498)

By Shin-ichi SHIMAMOTO and Kohnoshin ONODERA.

(Kyoto Imperial University; Received Apr. 28, 1939.)

On Translocation of Acid Radical of Glycerophosphoric Acid.

(pp. 499~502)

By Ryokoku YOKOYAMA.

(Kyoto Imperial University; Received Apr. 28, 1939.)

Hydroxylation of Sorbic Acid. I.

Synthesis of Talomethylose from Sorbic Acid.

(pp. 503~506)

By Masaru HAMADA and Toshitsune FUJITA.

(Kyoto Imperial University; Received Apr. 28, 1939.)

Über die chemischen Eigenschaft der einzelnen Teilen
der Getreidennischen Samen.

(pp. 507~513)

Von Tetsujiro OHARA.

(Tokyo Nogyo Kyoiku Senmon Gakko; Eingegangen am 27, Apr. 1939.)

Isolation of three Kinds of Isoflavon from Soya Bean.

(pp. 514)

By Koji OKANO and Iwao BEPPU.

(The Central Laboratory of the South Manchurian Railway Co., Received May 4, 1939.)

We obtained the following isoflavons from the by-product of the alcohol extraction of soya bean.

1. 8 methyl 5,4' dioxyisoflavon mp. 318 C.
2. 8 methyl 5, 7, 4' trioxyisoflavon mp. 298 C.
3. a glucoside of 5, 7, 2' trioxyisoflavon mp. 265 C.